Roles of regulatory T cells in enhancement of angiogenesis in a sponge implantation model

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Background: Angiogenesis is a process involved in several physiological/pathological events. Major players of angiogenesis are vascular endothelial cells, however non-endothelial cellular components such as macrophages and fibroblasts also regulate angiogenesis in vivo. In the present study, we used a sponge implantation model that can allow us to determine the angiogenesis quantitatively and clarified that regulatory T cells (Tregs) enhanced angiogenesis in vivo. Further, we clarified prostaglandins (PGs) generated by cyclooxygenase can enhance the angiogenesis via induction of Tregs in the site of angiogenesis.

Methods and Results: Treatment of mice with a CD25 neutralizing antibody reduced the granulation formation and CD31-positive structures in comparison with control treatment. CD31-positive vessel lumens were significantly low in the sections from the mice treated with a CD25 neutralizing antibody at day 14. CD31 mRNA levels were increased temporally, but the intraperitoneal injection of a CD25 neutralizing antibody reduced the expression levels to 70% at day 14. Foxp3-positive regulatory T cells recruited into the granulation tissues were markedly reduced with a CD25 neutralizing antibody at day 7 and day 14 with reduced expressions of vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β). Aspirin treatment reduced recruitment of Foxp3-positive regulatory T cells to granulation tissues with reduced angiogenesis. These reductions were accompanied with reduced expressions of VEGF and TGF-β.

Conclusions: The present study suggested that Treg had a proangiogenic activity in a sponge model and are target cells that regulate angiogenesis in pathological settings.

Key words: regulatory T cells (Tregs), angiogenesis, Aspirin
migration and activation. Angiogenesis is believed to be regulated by positive and negative regulators similar to that of the Th1/Th2 cells that modulate immune response. 

Regulatory T cells (Tregs) are another subtype of CD4 positive helper T cells, and are previously known as suppressor T cells. This subpopulation of T cells can modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune diseases. Tregs generally suppress or downregulate induction and proliferation of effector T cells. Tregs express biomarkers, such as CD4, FOXP3 and CD25, and are thought to be derived from the same lineage as naïve CD4 positive cells.

Tregs tend to be upregulated in individuals with cancer, and they seem to be recruited to the site of many tumors. Studies in both humans and animal models have implicated that high numbers of Tregs in the tumor microenvironment is indicative of a poor prognosis, and Tregs are thought to suppress tumor immunity, thus hindering the body’s innate ability to control the growth of cancerous cells. Angiogenesis is indispensable to tumor growth and is a target of cancer treatment. However, limited number of reports described that Tregs regulate tumor-associated angiogenesis, and the underlining mechanisms of enhancement of angiogenesis by Tregs are still controversial and poorly understood.

In the present study, we used a sponge implantation model that can allow us to determine the angiogenesis quantitatively and can mimic the proangiogenic tumor microenvironment. We clarified that Tregs enhanced angiogenesis in this model, and we revealed underlying mechanism of actions of Tregs to enhance angiogenesis. Further, we clarified PGs generated by cyclooxygenase can enhance the angiogenesis via induction of Tregs in the site of angiogenesis. The present study suggests that Tregs are target cells that regulate angiogenesis in the pathological settings.

Materials and Methods

Animals
Males C57 BL/6N mice (7 weeks old) were obtained from CLEA Japan Inc. (Tokyo, Japan). Animals were housed individually and maintained at constant humidity (60 ± 5 %) and temperature (25 ± 1°C) on a 12-h light/dark cycle. Mice were provided with food and water ad libitum. All experimental procedures were approved by the Animal Experimentation and Ethics Committee of the Kitasato University School of Medicine and were performed in accordance with the guidelines for animal experiments set down by the Kitasato University School of Medicine, which are in accordance with the "Guidelines for Proper Conduct of Animal Experiments" published by the Science Council of Japan.

Sponge implantation model
Circular sponge discs (5-mm -thick, 13-mm diameter, and 8.1 ± 0.3 mg, n = 10) were prepared from a sheet of polyether polyurethane foam by use of a wad punch. The discs were soaked in 80% ethanol for 3 hours and then rinsed in sterile distilled water. After air-drying, all discs were sterilized by a high-pressure steam sterilizer. Under light isoflurane (Wako Pure Chemical Industries, Ltd., Tokyo) anesthesia, they were then implanted the sponge discs into the subcutaneous tissue of their backs.

CD25 Neutralizing antibody treatment
A dose of 10 mg/kg body weight of anti CD25 (IL-2 receptor α chain) (Bio X Cell Co., West Lebanon, NH, USA) dissolved in physiological saline was intraperitoneally administered on the day before the start of the mouse experiment. For the control group, physiological saline was used.

Administration was carried out once a week, and administration experiments were conducted for 7 or 14 days.

Aspirin administration
A dose of 500 mg of gum arabic (Wako Pure Chemical Industries, Ltd.) was sufficiently pulverized in an agitated mortar and suspended in physiological saline. A dose of 100 mg of aspirin (Sigma-Aldrich, Tokyo) was overlaid thereon and thoroughly mixed in an agitator mortar to make 10 ml of the fine particle suspension with physiological saline. A dose of 100 mg/kg body weight was orally administered daily from the start date of the mouse experiment. A gum arabic suspension was used for the control group. At the time of administration, it was briefly anesthetized with isoflurane. Administration was continued for 7 or 14 days.

Tissue sample preparation
Mice were euthanized 7 or 14 days after the surgical sponge implantations. The back of the mice was dissected and the sponge granulation tissues and exudate were collected. After weighing the sponge granulation tissues, the exudate was sampled with a 1 ml syringe and was stored at -80°C. The sponge granulation tissues were divided in two. One piece was immediately fixed with 10% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4) and was soaked overnight for
pathological specimen preparation. Another piece was immersed in TRIzol Reagent (Thermo Fisher Scientific, Tokyo) for PCR quantification, and was homogenized and stored at -80°C.

Immunohistochemical analysis
The granuloma tissues in 10% paraformaldehyde were dehydrated in a graded ethanol series and then embedded in paraffin. Each section (3.5 μm) of the paraffin-embedded tissue was mounted on a glass slide and either stained with hematoxylin and eosin (H&E) or processed for immunohistochemistry. Pathological tissue specimens prepared were subjected to CD31, Foxp3, VEGF and TGF-β immunostaining.

For the latter, the sections were activated using Histo VT One (Nacalai Tesque, Yokohama) and then incubated overnight at 4°C with one of the following primary antibodies: (A) anti-mouse CD31 antibody (1:100, rabbit polyclonal, ab28364; Abcam, Cambridge, UK); (B) anti-mouse Foxp3 antibody (1:100, rabbit polyclonal, MA1-41628; Thermo Fisher Scientific Inc.); (C) anti-mouse VEGF-C1 antibody (1:200, rabbit polyclonal, sc7269; Santa Cruz Biotechnology, CA, USA); (D) anti-mouse TGF-β1 antibody (1:200, rabbit polyclonal, ab9758; Abcam, USA). For the primary antibodies, after immersion in a 3% solution of hydrogen peroxide (H2O2) for 30 minutes, the sections were incubated for 30 minutes at room temperature with N-Histofine Simple Stain Mouse MAX PO (Nichirei Bioscience, Tokyo) and were immersed in 0.02% 3,3'-diaminobenzidine and 0.3% nickel ammonium sulfate in 50 mM Tris-HCl buffer (pH 7.4) containing 0.005% H2O2 for 3 minutes. The specimens were counterstained with Mayer's Hematoxylin, dehydrated, cleared, and then mounted.

The images were captured with a light microscope (Biozero BZ-X710 Series; Keyence Corp., Osaka).

Enzyme-linked immunosorbent assay (ELISA) measurement
VEGF concentration in the exudate from the sponge was quantified by the enzyme-linked immunosorbent assay (ELISA) method. The ELISA kit was manufactured by R&D Systems, Minneapolis, MN, USA.

Real-time polymerase chain reaction (PCR) analysis
Transcripts encoding CD31, Foxp3, VEGF-A, TGF-β, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were quantified by real-time polymerase chain reaction (PCR) analysis. Total RNA was extracted from the granuloma tissues with TRIzol reagent, and single-stranded cDNA was generated from 1 µg of total RNA via reverse transcription with ReverTra Ace (Toyobo, Osaka). Quantitative PCR was performed with SYBR Premix Ex Taq (Takara Bio, Shiga). The real-time PCR primers were designed using Primer 3 software (http://primer3.sourceforge.net/) based on data from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and the following primers: 5'-ACTTCTGAACTCCAACAGCGA-3' (sense) and 5'-CCATGTTCTGCGGCTTATTAT-3' (antisense) for CD31, (sense) 5'-TACTCTCAAGTCCAAACATGCACACC-3', (antisense) 5'-CGCACAAGCACTTTGGCACTCAG-3' for Foxp3 5'-GAGAGGGCGGAAGGTCTTT-3' (sense) and 5'-TTGGAAC-CGGCATCTTTATC-3' (antisense) for VEGF-A. 5'-AAACATTCTCGGTGTTACCT-3' (sense) and 5'-TTATGCTCTCCTTGTTTCC-3' (antisense) for TGF-β1. For GAPDH, 5'-ACATCAGAAGGATGGTTAGC-3' (sense) and 5'-AAGGTGGAAGAGTGGGAGTTG-3' (antisense). Data were normalized to the level of GAPDH in each sample.

Statistical analyses
Data are expressed as means ± SD. All statistical analyses were performed using Graph Pad Prism software, version 5.01 (Graph Pad Software, La Jolla, CA, USA). Statistical comparisons between the two groups were calculated by Student's t-test, and P values <0.05 were considered to indicate statistical significance.

Results

Effects of a CD25 neutralizing antibody on granulation tissue formation and angiogenesis in sponge implants
One and 2 weeks after the implantation, granulation tissues were formed around the implants (Figure 1A). Intraperitoneal injections of a CD25 neutralizing antibody reduced the granulation formation at day 14 (Figure 1A). The cellular concentrations of the granulation tissues appeared sparse under a CD25 neutralizing antibody treatment (Figure 1B).

CD31 immunostaining revealed that CD31-positive structures appeared to be suppressed with injections of a CD25 neutralizing antibody at day 14 in comparison with control treatment (Figure 2A). When CD31-positive vessel lumens (red areas in Figure 2B) were quantified, the percentage of lumens in the observed cross section areas were significantly low in the sections from the mice treated with a CD25 neutralizing antibody at day 14 (Figure 2B, C). In real-time PCR analysis of CD31 in the granulation tissues isolated the sponge implants, CD31 mRNA levels were increased temporally (Figure 2D, open columns). Treatment with a CD25 neutralizing antibody
reduced the expression levels to 70% at day 7 (Figure 2D).

**Treatment with a CD25 neutralizing antibody reduced recruitment of Foxp3-positive regulatory T cells to granulation tissues in sponge implants.**

In the immunostaining of the granulation tissues, recruitment of Foxp3-positive regulatory T cells was identified (Figure 3A). The recruited cell number of Foxp3-positive cells appeared to be less in the sections under a CD25 neutralizing antibody treatment at day 14 (Figure 3A). When the cellular concentrations in the sections were quantified, the concentrations in Foxp3-positive regulatory T cells were markedly reduced with a CD25 neutralizing antibody at days 7 and 14 by 80% and 55%, respectively (Figure 3B). Real-time PCR for Foxp3 revealed mRNA levels were markedly reduced with a CD25 neutralizing antibody at days 7 and 14 by 30% and 58%, respectively (Figure 3C).

**Treatment with a CD25 neutralizing antibody reduced VEGF-positive cell accumulation, VEGF protein levels, and VEGF mRNA levels in granulation tissues in sponge implants**

In the immunostaining tested in the granulation tissues, the accumulation of VEGF positive cells was apparently suppressed with a CD25 antibody treatment at day 14 (Figure 4A). VEGF protein levels in the exudates in the sponge implants determined with ELISA were significantly lower in the samples treated with a CD25 neutralizing antibody at day 7 (Figure 4B). The mRNA levels of VEGF in the granulation tissues were also reduced with a CD25 neutralizing antibody at days 7 and 14 (Figure 4C).

**Treatment with a CD25 neutralizing antibody reduced TGF-β-positive cell accumulation and mRNA levels of TGF-β in sponge granulation tissues.**

The accumulation of TGF-β positive cells was also apparently suppressed at day 14 (Figure 5A). The mRNA

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**Figure 1.** Effects of a CD25 neutralizing antibody on sponge granulation tissue formation. (A) Weights of sponge implants isolated 1 and 2 weeks after the implantation. Mean ± SD. The number in parentheses is the number of mice tested. (B) H&E staining of the granulation tissues isolated 2 weeks after the implantation.
levels of TGF-β were also reduced with a CD25 neutralizing antibody at day 14 (Figure 5B).

**Effects of aspirin on granulation tissue formation and angiogenesis in the sponge implants**

To inhibit the endogenous formation of PGs, we administered aspirin from the day of the implantation throughout the experimental periods. One and 2 weeks after the implantation, granulation tissues had formed in/around the implants (Figure 6A). Daily oral administrations of aspirin reduced the granulation tissue formation at day 14 (Figure 6A). The cellular concentrations of the granulation tissues appeared sparse in/around the sponge implants (Figure 6B). CD31 immunostaining revealed that CD31-positive structures appeared to be suppressed with oral administration of aspirin at day 14 in comparison with vehicle treatment (Figure 7A). When CD31-positive vessel lumens (red area in Figure 7B) were quantified, the percentage of lumens in the observed cross section areas were significantly lower in the sections from the mice treated with aspirin at day 14 in comparison with mice treated with vehicle (Figure 7C). In real-time PCR analysis of CD31 in the granulation tissues isolated the sponge

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**Figure 2.** Effects of a CD25 neutralizing antibody on angiogenesis in sponge granulation tissues. (A) CD31 immunostaining at day 14. Arrowheads indicate CD31-positive structures. (B) CD31-positive vessel lumens (red areas) at day 14. (C) The percentage of lumens in the observed cross section areas at day 14. Mean ± SD. The number in parentheses is the number of mice tested. (D) Real-time PCR analysis of CD31 in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.
Figure 3. Reduced recruitment of Foxp3-positive regulatory T cells to granulation tissues with a CD25 neutralizing antibody treatment. (A) Immunostaining of Foxp3 at day 14. Arrowheads indicate Foxp3-positive structures. (B) Concentrations in Foxp3-positive regulatory T cells in the cross sections. Mean ± SD. The number in parentheses is the number of mice tested. (C) Real-time PCR for Foxp3. Mean ± SD. The number in parentheses is the number of mice tested.

Figure 4. VEGF-positive cell accumulation, VEGF protein levels and VEGF mRNA levels in granulation tissues after treatment with a CD25 neutralizing antibody. (A) Immunostaining of VEGF at day 14. (B) VEGF protein levels in the exudates in the sponge implants determined with ELISA. Mean ± SD. The number in parentheses is the number of mice tested. (C) mRNA levels of VEGF in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.
Figure 5. TGF-β-positive cell accumulation, and TGF-β mRNA levels in granulation tissues after treatment with a CD25 neutralizing antibody. (A) Immunostaining of TGF-β at day 14. (B) mRNA levels of TGF-β in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.

Figure 6. Effects of aspirin on granulation tissue formation. (A) Weights of sponge implants under aspirin treatment isolated 1 week and 2 weeks after the implantation. Mean ± SD. The number in parentheses is the number of mice tested. (B) Typical H&E staining of granulation tissues formed around the implants at day 14.
implants, CD31 mRNA levels were increased temporally (Figure 7D, open columns). Treatment with aspirin reduced the expression levels to 60% at day 14 (Figure 2D). In the immunostaining tested in the granulation tissues, CD31-positive structures, (Figure 7A) and vascular lumen percentage was suppressed with aspirin at day 14 (Figure 7B, C). The same was true in CD31 mRNA levels at day 14 (Figure 7D).

Aspirin treatment reduced recruitment of Foxp3-positive regulatory T cells to sponge granulation tissues. Recruitment of Foxp3-positive regulatory T cells were apparently suppressed with aspirin at day 14 (Figure 8A). When the cellular concentrations in the sections were quantified, the concentrations in Foxp3-positive regulatory T cells were significantly reduced with aspirin at days 7 and 14 by 35% and 55%, respectively (Figure 8B). Real-time PCR for Foxp3 revealed mRNA levels were markedly reduced with aspirin at day 14 by 40% (Figure 8C).

Treatment with aspirin reduced expressions of VEGF and TGF-β in granulation tissues in sponge implants. Aspirin treatment reduced accumulation of VEGF

Figure 7. Effects of aspirin on angiogenesis in sponge implants. (A) CD31 immunostaining at day 14. Arrowheads indicate CD31-positive structures. (B) CD31-positive vessel lumens (red areas) at day 14. (C) The percentage of lumens in the observed cross section areas at day 14. Mean ± SD. The number in parentheses is the number of mice tested. (D) Real-time PCR analysis of CD31 in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.
Figure 8. Reduced recruitment of Foxp3-positive regulatory T cells to granulation tissues with aspirin treatment. (A) Immunostaining of Foxp3 at day 14. Arrowheads indicate Foxp3-positive structures. (B) Concentrations in Foxp3-positive regulatory T cells in the cross sections. Mean ± SD. The number in parentheses is the number of mice tested. (C) Real-time PCR for Foxp3. Mean ± SD. The number in parentheses is the number of mice tested.

Figure 9. VEGF-positive cell accumulation, VEGF protein levels and VEGF mRNA levels in granulation tissues after aspirin treatment. (A) Immunostaining of VEGF at day 14. (B) VEGF protein levels in the exudates in the sponge implants determined with ELISA. Mean ± SD. The number in parentheses is the number of mice tested. (C) mRNA levels of VEGF in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.
positive cells in the granulation tissues at day 14 (Figure 9A). VEGF protein levels in the exudates in the sponge implants determined with ELISA were significantly lower in the samples treated with aspirin at days 7 and 14 (Figure 9B). The mRNA levels of VEGF were also reduced with aspirin at day 14 (Figure 9C).

The accumulation of TGF-β positive cells was also apparently suppressed with aspirin at day 14 (Figure 10A). The mRNA levels of TGF-β were also reduced with aspirin at days 7 and 14 significantly (Figure 10B).

Discussion
In the present study, we clarified that angiogenesis seen in the granulation tissues formed around the sponge implants was dependent on the accumulation and/or function of Tregs. The recruited Tregs induced VEGF and TGF-β in the sponge proangiogenic microenvironment. Further, endogenous PGs, potent proangiogenic factors in this sponge model, induced Treg-recruitment to the site of angiogenesis and induced VEGF and TGF-β. These suggested that Tregs were potent regulators of angiogenesis in vivo.

Angiogenesis is a process to make a functional active vasculature from the preexisting vascular beds. Several growth factors were reported to enhance the proangiogenic responses. We had repeatedly reported that arachidonic acid metabolites, PGs enhanced angiogenesis during tumor growth,8 and wound healing.14 Major players of the angiogenesis were no doubt the endothelial cells,15 but other cellular components were quite active in the pathological settings. PGs act on the cellular components of proangiogenic microenvironments, such as tumor stromal tissues, wound granulation tissues, and chronic inflammation sites. Quite recently, a subtype of CD4 positive helper T cells, Tregs have been reported to modulate angiogenesis in some pathological settings.13,16-21

In a tumor microenvironment, characterized by a

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**Figure 10.** TGF-β-positive cell accumulation, and TGF-β mRNA levels in granulation tissues after treatment with aspirin. (A) Immunostaining of TGF-β at day 14. (B) mRNA levels of TGF-β in the granulation tissues. Mean ± SD. The number in parentheses is the number of mice tested.
multitude of mechanisms supporting angiogenesis and immune suppression, Tregs are considered to be pivotal mediators of peripheral tolerance and immune suppression. Tregs are highly enriched in the tumor microenvironment and are well known for their roles in tumor progression. Recently, Facciabene et al.\textsuperscript{19} reported the roles of Tregs beyond immune suppression in tumors and have demonstrated that Tregs are directly involved in promoting angiogenic reprogramming of the tumor microenvironment,\textsuperscript{22} highlighting a multifaceted role for Tregs in promoting cancer through angiogenesis besides tumor immune escape. Müller-Hermelink et al.\textsuperscript{21} and Qin et al.\textsuperscript{24} reported that Tregs promoted angiogenesis by suppressing the activities of Th1 effector T cells releasing angiostatic cytokines like TNF-\( \alpha \) and IFN-\( \gamma \), as well as interferon-induced chemokines, such as CXCL9, 10, and 11. It was also reported that Tregs have been shown to promote tumor angiogenesis by specifically inhibiting tumor-reactive T cells.\textsuperscript{25} However, Casares, et al.\textsuperscript{25} did not demonstrate that Tregs could make significant contributions to the direct promotion of tumor angiogenesis. Tumor stromal reactions were modified by the tumor cell profiles. The sponge models, we used in the present study, may provide more direct data of Treg contributions without noises affected by tumor cell properties. We identified that Tregs exhibited proangiogenic activities via upregulation of VEGF and TGF-\( \beta \) in the granulation tissues. The accumulation of Tregs at tumor sites has been correlated with biomarkers of accelerated angiogenesis such as VEGF overexpression and increased microvessel density in endometrial\textsuperscript{26} and breast cancers.\textsuperscript{27} These are quite consistent with the results from present results, highlighting the importance of VEGF as a down stream molecules relevant to Treg accumulation. Tregs, secreted large amounts of TGF-\( \beta \) that inhibited CD8\(^+\) effector T cell functions \textit{in vitro}.\textsuperscript{28} In the present study, we identified up regulation of TGF-\( \beta \) in the granulation tissues. Importantly this up regulation was hampered with CD25 neutralizing antibody. Since TGF-\( \beta \) increased angiogenesis and Treg function, the positive feedback mechanisms may be involved in the present Treg-dependent enhancement of angiogenesis.

When ischemia was induced by right femoral artery ligation, angiogenic response was markedly enhanced in CD28-deficient mice, in which the number of Tregs was reduced compared with wild type controls.\textsuperscript{19} Similarly, anti-CD25 treatment and subsequent Treg deletion significantly enhanced postischemic neovascularization.\textsuperscript{19} These effects were associated with enhanced accumulation of CD3-positive T cells and Mac-3-positive macrophages in the ischemic leg. These results were not consistent with the results in the sponge implantation models. Treatment of CD28\(^+\) mice with the non-mitogenic anti-CD3 monoclonal antibody enhanced the number of endogenous Treg cells and led to a significant reduction of the postischemic inflammatory response and neovascularization.\textsuperscript{19} Contrastingly, the contribution of Tregs in responses to hind limb ischemia is reported to be minimum.\textsuperscript{29} Further studies are warranted for this ischemia model.

The same was true in the brain ischemia model. The results from the previous studies are controversial. Liesz et al.\textsuperscript{30} and Kleinschnitz et al.\textsuperscript{31} showed that Treg cells exacerbated brain injury early after transient ischemia, whereas Ren et al.\textsuperscript{18} and Li et al.\textsuperscript{32} reported T-cell therapy protected against cerebral ischemia. The discrepancies in these results could be derived from the different methods that were used in these studies. Thus, new studies are necessary to evaluate the potential therapeutic usefulness of Tregs in brain ischemia.

We had repeatedly reported that endogenous PGs enhanced angiogenesis during wound healing\textsuperscript{44} and tumor growth.\textsuperscript{8,33,34} PGs actions were mediated with G-protein coupled receptors. Many of the PG actions are immediate ones, such as smooth muscle contractions, hypotension, and platelet aggregations. But PGs also participate in inducing the gene expressions of various kinds of cytokines, chemokines, and growth factors. In the present study we showed that when PGs’ biosynthesis was blocked with aspirin, the recruitment of Tregs were suppressed. To our knowledge, this is the first finding that PGs regulate recruitment of Tregs in the proangiogenesis in an angiogenic microenvironment.

The present sponge model is an artificial one, however this study provides evidence that Tregs enhance angiogenesis \textit{in vivo}. In conclusion, we clarified that Tregs exhibited proangiogenic activity. Treatment with a CD25 neutralizing antibody reduced the granulation formation and angiogenesis. CD31-positive vessel lumens were significantly low in the sections from the mice treated with a CD25 neutralizing antibody. Aspirin treatment reduced recruitment of Foxp3-positive regulatory T cells to granulation tissues with reduced angiogenesis. These reductions were accompanied with reduced expressions of VEGF and TGF-\( \beta \). The results suggested that Tregs had proangiogenic activities in the sponge model and are target cells that regulate angiogenesis in pathological settings.
References


Regulatory T cells and angiogenesis


